Signalling assemblies: the odds of symmetry

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Abstract

The assembly of proteins into complexes is fundamental to nearly all biological signalling processes. Symmetry is a dominant feature of the structures of experimentally determined signalling complexes, observed in the vast majority of homomers and many heteromers. However, some asymmetric structures exist, and asymmetry also often forms transiently, intractable to traditional structure determination methods. Here, we explore the role of protein complex symmetry and asymmetry in cellular signalling, focusing on receptors, transcription factors and transmembrane channels, amongst other signalling assemblies. We highlight a recurrent tendency for asymmetry to be crucial for signalling function, often being associated with activated states. We conclude with discussion of how consideration of protein complex symmetry and asymmetry has significant potential implications and applications for pharmacology and human disease.

Introduction

Many if not most proteins can assemble into complexes in order to carry out their biological functions. The three-dimensional structures of tens of thousands of protein complexes have been experimentally determined to date, and have revealed a tremendous diversity of possible quaternary structure, *i.e.* the way the different subunits of a complex are arranged with respect to each other [1]. However, while the importance of protein interactions is widely recognised, the implications of higher-order quaternary structure are often not considered when attempting to understand protein function and malfunction.

Protein complexes can be divided into homomers, formed from multiple copies of the same protein, and heteromers, containing multiple distinct proteins. Recently, it has been shown that much of the diversity of protein quaternary structure observed in nature can be explained by a simple model based upon the possible transitions through which protein complexes can evolve, and which allows most known structures to be arranged into a “periodic table of protein complexes” [2]. Symmetry is a defining feature of this periodic table, as it allows the grouping of heteromeric complexes with topologically equivalent homomers from the same symmetry group. Approximately 96% of homomer structures can be classified into a limited set of closed symmetry groups [3,4]. For heteromers, if we exclude the 65% of structures that have no repeated subunits (*e.g.* heterodimers), then 79% are symmetric. Of those homomer or heteromer structures that are asymmetric, the majority are the result of quaternary structure assignment errors [2,5], or appear symmetric under equilibrium conditions in solution [6].

Despite the prevalence of symmetry in protein complex structures, biological asymmetry is common. In fact, since the early days of protein crystallography, with the observation that many proteins form symmetric complexes, the role of asymmetry has been discussed. While the Monod-Wyman-Changeux (MWC) model of allostery relies on the preservation of global symmetry [7], the subsequent Koshland-Némethy-Filmer (KNF) model is dependent upon the simultaneous adoption of distinct conformations by different subunits within the same complex [8]. The

asymmetry allowed by the KNF model can explain the negative cooperativity observed in some proteins, which is not accounted for by the MWC model [9].

Asymmetry in protein complexes can generally occur in two different ways. First, there are protein complexes that have their structures determined in a genuinely asymmetric state. If we exclude heteromers with no repeated subunits (*i.e.* 1:1 stoichiometry), then For instance, complexes with uneven (odd) stoichiometry (which comprise ~25% of heteromeric structures with repeated subunits) necessarily contain some degree of asymmetry as sequence-identical subunits must form different interactions within the complex [2,10]. Second, asymmetry is often transient and unobservable to traditional structure determination methods, but detectable using various biochemical and biophysical techniques. Interestingly, asymmetric states of protein complexes are often conducive to the biological functions of signalling complexes, as we will discuss in this review.

A variety of different biophysical methods are available to address symmetry-related issues, and their strengths and limitations have been recently compared [11]. X-ray crystallography has remained a major method to reveal atomic differences between subunits of complexes and thus asymmetry, but is limited by the difficulty of crystallising many proteins, and the fact that it presents only a static snapshot of protein structure. Due to methodical advances, single particle cryo-electron microscopy has recently reached near-atomic resolution and is quickly becoming a major method to elucidate distinct (a)symmetric states of large complexes [12]. The shape, assembly state of these large complexes in solution can be confirmed with small-angle X-ray or neutron scattering. Mass spectrometric methods have increasingly become more efficient and complementary to determine the molecular weight and thus the stoichiometry of large assemblies in solution [13].

One important caveat to consider when analysing X-ray or electron microscopy structures is that symmetry constraints are often utilised, which essentially assume that symmetry is present and that different copies of the same subunit are in identical conformations [14]. While such symmetry constraints are currently necessary to obtain a structure solution in many cases, particularly for large structures where the resolution is poor, they have the potential to mask small or localised asymmetry.

The detection of the shortest-lived events needs site-specific labelling with paramagnetic atoms, fluorescent or luminescent markers based on stereo-structural knowledge. Single molecule fluorescence and bioluminescence resonance energy transfer measurements have outstanding potential to reveal the temporal and structural details of activation. Nuclear magnetic and electron paramagnetic resonance spectroscopies in solid state and solution, respectively, can be used to probe local conformation and rapid structural changes. Finally, molecular dynamics simulations can mimic asymmetric transition states approaching the μs timescale. Complementary combinations of these methods with biochemical and nanotechnological ones can elucidate the asymmetric activation of signalling complexes more and more reliably [15].

In this review, we discuss the role of protein complex symmetry and asymmetry in protein complexes involved in signalling processes. In particular, we focus on GPCRs, other dimeric receptors and transcription factors, and transmembrane channels and transporters, showing that, while many of these complexes have symmetric structures, asymmetry is often important for their function and regulation. An overview of some of the main examples discussed in this review is provided in Table 1. We also consider the implications of protein complex (a)symmetry for pharmacology and in understanding the molecular mechanisms of human disease. Finally, through a simple analysis of known protein quaternary structures, we highlight an overrepresentation of both symmetric and asymmetric complexes amongst proteins known to be drug targets or associated with genetic disease or cancer.

Multiple levels of symmetry and asymmetry in G protein-coupled receptors

G protein-coupled receptors (GPCRs) are the largest group of drug targets and serve as a challenge for the development of allosteric modulators and GPCR subtype-selective drugs exploiting the heterogeneity of signalling [16–19]. Recent advances in X-ray crystallography have led to successful determination of several structures of GPCR transmembrane regions. While most structures are monomeric, proteins in three different GPCR classes have been crystallised as symmetric homodimers [20–22]. However, despite this symmetry, there is considerable evidence that transient asymmetry can occur between the dimeric subunits upon interaction with other

proteins or small molecules. For example, oligomeric states of various GPCRs have been detected in native tissues using fluorescence resonance energy transfer [23], and there appears to be asymmetry between the dimer subunits in a short-lived intermediate state [24]. Other studies have highlighted asymmetry in the ligand-activated states of metabotropic glutamate receptors (mGluRs) [25], agonist-bound leukotriene B4 receptors [26], serotonin 5-HT2c receptors [27] and dopamine D1-D2 receptors [28,29]. Some GPCRs, such as dopamine D2 and $GABA_B$ receptors, can form pseudosymmetric heterodimers, and this pseudosymmetry can be broken upon transactivation [30–33].

Only a single structure of a GPCR in complex with a heterotrimeric G protein has been published [34]. Although it involves a monomeric GPCR (β2 adrenergic receptor) and so has even (1:1:1:1) stoichiometry (Figure 1A), a homodimeric GPCR can also activate G proteins with asymmetric uneven stoichiometry [35–37]. For example, one regulator of G-protein signalling and one G_i can bind asymmetrically to separate protomers of a melatonin receptor dimer that rearranges upon agonist activation [38]. Similarly, a single C-terminal domain of GPCR and (rhod)opsin dimers binds intracellular regulatory proteins such as arrestin [39–41]. Finally, a recent analysis of the proteome of native $GABA_B$ receptor signalling has revealed uneven stoichiometries where the core assemblies contain $GABA_{B1a/b}$, $GABA_{B2}$, four channel tetramerisation domains and distinct G protein subunits [42].

Higher-order structures are also possible for GPCRs [37]. For example, the extracellular domain of follicle-stimulating hormone (FSH) receptor assembles into a symmetric homotrimer, and can form an even (3:3:3) stoichiometry complex with FSH α and ß [43]. Upon interaction with a heterotrimeric G protein, an asymmetric uneven stoichiometry complex may be formed [44]. Although no structure is available for this full complex, we can combine available structures with our knowledge of the stoichiometry to build a speculative model of this 3:3:3:1:1:1 complex (Figure 1A). There is also evidence that GPCRs can exist as homotetramers and heterotetramers [45,46], which can allosterically influence the potencies and efficacies of agonists [18,19,47]. Examination of crystal packing in dimers provided possible structures of GPCR tetramers [35,48,49]. Due to the non-equivalent positions of subunits, these tetramers are asymmetric, or “non-bijective” according to the nomenclature of the periodic table of protein complexes [2]. Although a majority of non-bijective homomer

structures are the result of quaternary structure assignment errors [2], they can nevertheless be used to putatively model how a GPCR tetramer can simultaneously bind two different heterotrimeric G proteins with uneven (4:2:2:2) stoichiometry [35] (Figure 1A). Finally, rhodopsin dimers have been observed to form higher-order assemblies in native disc membranes [50].

Dimeric receptors and transcription factors: activation and breaking of twofold symmetry

In addition to GPCRs, there are many other signalling proteins that form homodimers, *e.g.* transmembrane receptors like receptor tyrosine kinases, and transcription factors, including nuclear receptors. While most of the known structures of these proteins have twofold symmetry, asymmetry is often important for their activation and function, in particular due to the uneven stoichiometry of their interactions with ligands or other proteins. Any interaction with uneven stoichiometry will necessarily be asymmetric, unless the ligand itself is symmetric [10]. For example, asymmetric 2:1 complexes have been observed for a variety of receptor-ligand complexes, as we illustrate for interleukin 17A [51] and prolactin [52] in complex with their receptors (Figure 1B). In these structures, since the ligand binds at or near the dimer interface, the two receptor subunits will necessarily interact in different ways. Other receptor-ligand complexes with evidence for 2:1 stoichiometry when activated include bone morphogenic protein [53], insulin [54], fibroblast growth factor [55] and mammalian target of rapamycin (mTOR) kinase [56].

For dimeric transcription factors, symmetry is often broken in a similar way, due to the interaction of a symmetric dimer with asymmetric double-stranded DNA. Thus, asymmetry is often observed in the DNA-bound state of transcription factors. For example, the homodimeric retinoid X receptors bind co-operatively but asymmetrically to DNA repeats [57] (Figure 1B). Similarly, diabetes-related hepatocyte nuclear transcription factors homodimerise and form an asymmetric complex with the DNA response element [58] (Figure 1B). The heat-shock factor HSF1 presents a particularly interesting case: upon stress, it can trimerise via its coiled-coil domain and wraps around its DNA in an asymmetric manner [59]. In contrast, some transcription factors bind at palindromic sequences or inverted

repeats [60], which have local twofold symmetry, and thus symmetry can be preserved in the DNA-bound state [61].

Asymmetry can be also observed at the level of the dimer alone, such as the cytoplasmic kinase domain of epidermal growth factor receptor (EGFR), which was crystallised as a symmetric dimer in its autoinhibited state, but as an asymmetric dimer in its activated form [62] (Figure 1B), showing that asymmetric dimer rearrangement is essential for kinase activation. Similarly, the cytoplasmic region of the bacterial receptor histidine kinase CpxA crystallised as an asymmetric dimer, suggesting that chemotaxis signalling is a highly dynamic process that occurs via asymmetric rearrangement of the catalytic domains [63]. Although there are no mammalian orthologues, the bacterial histidine kinases are emerging as potential antibiotic drug targets [64].

The aberrant (a)symmetry of dimeric signalling complexes is often intimately associated with pathogenesis, particularly cancer. For example, disease-related mutations in the dimer interfaces of kinase domains can potentially impair the activation of various growth factor receptors and kinases [65]. Mig6 and anticancer drugs inhibit EGFR and drive internalisation via uneven (2:1) stoichiometry complexes, while oncogenic mutants may form even (2:2) stoichiometry complexes with Mig6 [66]. Tyrosine kinase inhibitors can enhance symmetric or pseudosymmetric EGFR interactions, thus restructuring the network of EGFR interactions [67]. An oncogenic missense mutation of the fibroblast growth factor receptor FGFR4 gene exposes a binding site for STAT3, a signal transducer and activator of transcription, which alters the stoichiometry and enhances STAT3 signalling [68]. Haem-dependent symmetric dimerisation of sigma-2 receptors facilitates cancer proliferation and chemoresistance [69].

Transient and permanent asymmetry in oligomeric transmembrane channels and transporters

Ligand- and voltage-gated ion channels are activated by chemical and physical signals. Although these transmembrane channels typically have cyclic symmetry or pseudosymmetry [70,71], asymmetry often occurs through their interactions with other proteins. For example, skeletal muscle ryanodine receptors are 4-fold

symmetric homotetramers [72] (Figure 1C) that can interact asymmetrically via their C-terminal intracellular domains with other integral membrane proteins, such as the 9-fold symmetric caveolin-3 [73]. Similarly, cardiovascular K_{ATP} and voltage-activated (Shaker) Kv potassium channels are 4-fold symmetric [74], and their clustering requires binding of the C-terminal disordered domains to PSD-95 scaffold proteins, presumably with uneven stoichiometry [75]. Finally, purinergic P2X7 receptor trimers form uneven stoichiometry complexes with symmetric pannexin-1 channels involved in cardioprotection [76].

Some transmembrane channels are pseudosymmetric heteromers with paralogous subunits, presumably having evolved from symmetric homomers via gene duplication [77]. If the ancestral homomer has an even number of subunits, then even stoichiometry can be maintained after gene duplication. However, gene duplication will cause a cyclic ring with an odd number of subunits to evolve with uneven stoichiometry. For example, members of the Cys-loop superfamily of neurotransmitter receptors are pentameric ligand-gated ion channels (pLGIC) fine-tuned by allosterically modulating drugs [78]. Bacterial pLGICs are symmetric homopentamers [79,80], while most mammalian pLGICs are pseudosymmetric heteropentamers with uneven stoichiometry [81,82]. Distinct auxiliary subunits confer tissue selectivity, such as in muscle-type pseudosymmetric α_2ßγδ nicotinic acetylcholine receptors [76] (Figure 1C). Activation of Cys-loop receptors requires agonist binding in three cavities of non-consecutive subunit interfaces [83,84], thus breaking pseudosymmetry [78,85]. In contrast, ligand occupation of all five binding clefts restores pseudosymmetry, resulting in desensitisation and channel closure [85]. Interestingly, rescue of truncated pLGIC function by domain complementation needs inter-familial co-assembly and thus uneven stoichiometry [86].

The homotetrameric crystal structure of the antagonist-bound AMPA-type GluA2 ionotropic glutamate receptor (iGluR) revealed an interesting case of mixed symmetries: the transmembrane region has 4-fold cyclic symmetry, while the extracellular domains form a pair of symmetric dimers [87]. The agonist-bound active form showed conformational changes leading to deeper expansion of the twofold symmetry of the extracellular domains and increased tension in the linkers connecting ligand-binding domain to the N-terminal and transmembrane domains [88]. When activated by a homodimeric cone snail toxin, GluA2 forms an uneven

(4:2) stoichiometry complex where asymmetric constraints by the toxin across the ligand-binding domain force the opening of the channel [89] (Figure 1C). AMPA-type iGluR homotetramers co-assemble with a regulatory protein stargazin, mostly with uneven (4:3) stoichiometry [90]. When these complexes bound antagonists, they showed twofold symmetry [91]. Finally, all types of iGluRs (AMPA, kainite and NMDA) can form paralogous heterotetramers with global twofold symmetry, as well as twofold pseudosymmetry in the extracellular domains and 4-fold pseudosymmetry in the transmembrane region [92–94] (Figure 1C).

While signal-activated transmembrane channels enable the flux of chemicals down their concentration gradients, active transporters are needed to expel undesired substances or accumulate necessary ones. Many membrane transporters are also symmetric oligomers [71]. Some transporters undergo rapid symmetry-violating transitions between outward- and inward-facing conformations, around twofold and 3-fold pseudosymmetric assemblies with even stoichiometries and/or intramolecular inverted repeats [95]. However, auxiliary subunits result in further asymmetry. The ATP-driven TrkH belongs to the superfamily of K^+ transporters. TrkH dimers and ATP-bound TrkA tetramers form symmetry-breaking uneven stoichiometry assemblies [96]. At the active mitochondrial protein gate the preprotein-translocating trimeric complex of rings reassembles into dimeric translocator of the outer membrane (TOM) rings [97].

Many pathogenic mutations have been associated with symmetric transmembrane channels and these “channelopathies” are challenging targets for selective drugs [98–100]. Interestingly, the mutations are often autosomal dominant, and thus asymmetric at the gene level (i.e. heterozygous). Thus, these mutations can break the symmetry of complexes, as they will assemble with a mixture of wild type and mutated subunits. This phenomenon allows for a dominant-negative mechanism, assuming that all complexes containing at least one mutated subunit experience a loss of function [101], and assembly does not occur co-translationally [102,103]. In the example shown in Figure 2, 1/16 of the assembled complexes will be C_4 symmetric wild-type homomers, 1/16 will be C_4 symmetric mutant homomers, 2/16 will be C_2 symmetric heteromers and 10/16 will be asymmetric heteromers. Interestingly, however, dominant-negative mutations in transmembrane channels tend to be less structurally destabilizing than recessive or other dominant mutations,

as the dominant-negative mechanism requires that the complex is still able to assemble [104].

Pharmacological implications of symmetry and asymmetry

We have discussed a number of pharmacologically relevant complexes with evidence for transient or permanent asymmetry. However, beyond simply knowing that many signalling complexes are often symmetric or asymmetric, how can knowledge and understanding of the principles of protein complex symmetry and asymmetry help pharmacology?

Perhaps most importantly, there appears to be a regular tendency for symmetry to be associated with inactive states and asymmetry to be associated with activation. This has important pharmacological implications. Pharmacological blockade by antagonists requires stabilisation of an inactive state, which could involve interface binding to strengthen symmetric homomeric interactions, or to prevent asymmetric, usually heteromeric interactions. Symmetry considerations can even be extended to the small-molecule level: symmetric organic anions and calixarenes can match the symmetry and inhibit homomeric pLGICs [105] and voltage-dependent K_v channels [106]

In contrast, agonist-elicited signal transductions either weaken initial interface interactions or foster subsequent subunit attachments which can result in asymmetric reassembly. This is illustrated by product-elicited complex reassembly of arginine methyltransferase with its coactivator CARM [107]. In addition, a bacterial homopentameric pLGIC model of mammalian $GABA_A$ receptors revealed that potentiation by sedative benzodiazepine drugs needs asymmetric interface binding of agonists and allosteric agents for channel opening [108]. Glycine and $GABA_A$ receptor concatemers with constrained stoichiometry also demonstrated asymmetric contributions of subunits to activation [109], and potentiation by anaesthetic alcohols (e.g. propofol) needs asymmetric binding patterns in transmembrane cavities [79,85].

Some viral antigenic peptides are transported into the endoplasmic reticulum by a transporter associated with antigen processing (TAP) and then onto the major histocompatibility complex. An inhibitory protein of herpes simplex virus can asymmetrically bind and stabilise the pseudosymmetric TAP dimer in a cytosol-facing

state, which is a valuable tool for selective immunosuppression [110]. Any inhibitor working via a similar mechanism to “plug” a (pseudo)symmetric transporter or channel would necessarily require a similar asymmetric mode of binding, or else be symmetric itself to match the symmetry of complex.

Antibody design can be aided by symmetry considerations. For example, antigen binding induces the symmetric hexamerisation of IgG, which allows the formation of activating complexes with C1, the first component of complement [111,112]. In addition, recent reports describe the structures of neutralizing antibodies forming 3-fold symmetric complexes with the envelope glycoprotein trimers of HIV and Ebola viruses [113–115].

Finally, it is important to consider the fact that asymmetry can sometimes lead to counterintuitive pharmacological effects. For example, identical subunits within the same complex can behave in different ways, as seen in asymmetric GPCR dimers where agonists can bind to different subunits with different affinities and efficacies [18,116].

Analysis of quaternary structure supports the pharmacological and biomedical importance of symmetry and asymmetry

Another way to illustrate the biomedical and pharmacological importance of quaternary structure is with a structural bioinformatic approach. The huge number of protein structures now available allows to classify human protein-coding genes on the basis of their quaternary structure: whether they are known to form a symmetric or asymmetric homomer or heteromer, or whether they are monomeric with no evidence of complex formation. We used these classifications to investigate how frequently different types of protein quaternary structures are associated with human protein-coding genes that are known to be drug targets [117], associated with a Mendelian genetic disease [118], or associated with cancer [119] (Figure 3). Importantly, we emphasise that the fact that proteins are drug targets or associated with genetic disease or cancer are not independent of each other - this allows us to highlight the potential role of symmetry and asymmetry in biomedically relevant proteins.

Overall, the most striking observation is that human proteins that assemble into complexes are significantly more likely to be drug targets or be associated with genetic disease or cancer than monomeric proteins. The difference between symmetric and asymmetric structures is small, but this is confounded by the fact that asymmetric structures are often the result of quaternary structure assignment errors [2], and that symmetric complexes can adopt transient asymmetry. However, symmetric structures do appear to have a slightly stronger tendency to be drug targets or be associated with genetic disease. For example, symmetric heteromers are significantly more likely to be drug targets than asymmetric heteromers (P = 0.006, Fisher's exact test).

For the most part, there is little apparent difference between homomers and heteromers - both are similarly enriched as drug targets and in genetic disease compared to monomers. The exception is the cancer-associated genes, where the subunits of heteromers are highly enriched compared to homomers ($P = 2 \times 10^{-7}$, when heteromeric and homomeric subunits are grouped). This may reflect the tendency for cancer genes to be involved in signalling pathways requiring interactions between distinct proteins [120].

There are a number of potential caveats related to this simple approach. In particular, quaternary structure can be dynamic, *e.g.* a single protein may exist as a monomer or as a part of different homomers and heteromers. Furthermore, different quaternary structures may be associated with different biological functions [121], *e.g.* transmembrane channels often have cyclic symmetry [71], and allosteric enzymes are often dihedral [3]. Since transmembrane channels are often drug targets and metabolic enzymes are often associated with Mendelian genetic disorders, the associations we see may reflect the fact that different forms of oligomerisation can facilitate different biological functions. Finally, the low association of proteins without published structures with all three groups is probably due to the fact that proteins of biomedical interest are more likely to have been studied experimentally. Nevertheless, this analysis supports the general importance of proteins that assemble into symmetric and asymmetric complexes, as they are more likely to be drug targets or associated with disease.

Conclusions and perspectives

Major advances have been made in recent years in our ability to experimentally characterise symmetric and asymmetric quaternary structure. Although X-ray crystallography has revealed symmetric structures for thousands of protein complexes, this has led to a somewhat artificial sense of the dominance of symmetry. As we have shown here, asymmetry is probably more common than the static crystallographic picture reveals, and the importance of asymmetry to dynamic cellular processes is becoming increasingly clear. In particular, we see a frequent correlation between symmetry breaking and biological function or activation. However, since these asymmetric, active states are often higher energy and transient, they are more difficult to detect experimentally. Instead, we must often rely on indirect methods to observe or infer the presence of asymmetry. Future improvements in these techniques, integrated with detailed molecular simulations and improved knowledge of the principles that underlie symmetric and asymmetric quaternary structure organisation [2,10], will allow us to understand the full spectrum of symmetric and asymmetric states associated with various signalling processes occurring within cells, including the emerging concept of hierarchical, dynamic signalling assemblies called signalosomes [122].

Symmetry is a unifying concept in a broader sense [123]. It has remained largely unaddressed in pharmacological studies and constrained in structural biology. We still lack a complete picture of the role of asymmetry in cellular signalling, and we cannot answer the majority of basic questions unambiguously yet. Better consideration of symmetry and asymmetry will improve both our understanding of cellular signalling processes at a molecular level, and our ability to target them pharmacologically.

Acknowledgments

We thank György Abrusán, Therese Bergendahl, Dinesh Soares and Jonathan Wells for helpful comments on the manuscript. J.A.M. is supported by a Medical Research Council Career Development Award (MR/M02122X/1).

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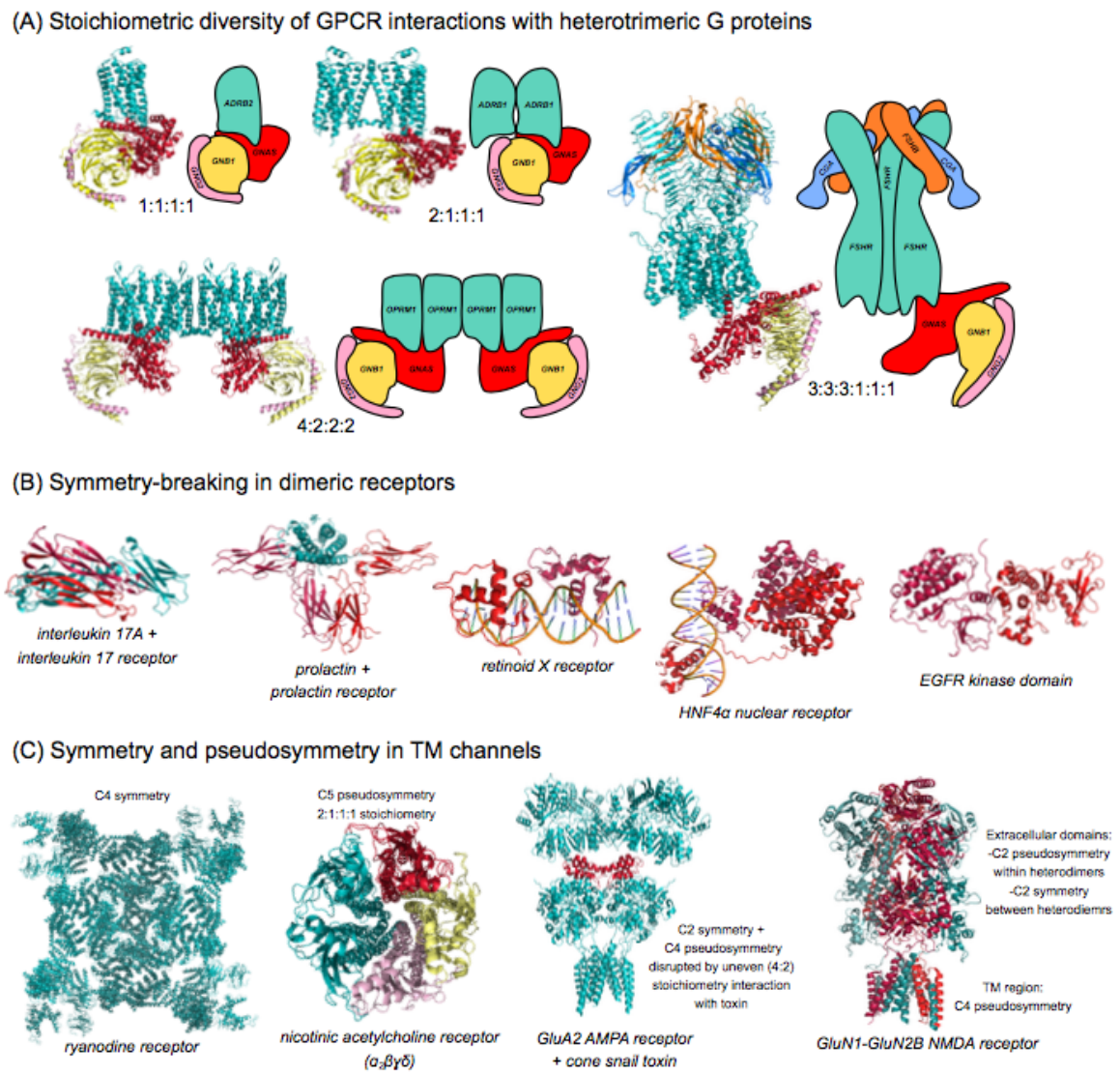

Figure 1: Symmetry and asymmetry in the structures of signalling complexes.

(A) Structure of a GPCR in complex with a heterotrimeric G protein, having even (1:1:1:1) stoichiometry (PDB ID: 3SN6), and putative models of complexes of other GPCRs forming uneven stoichiometry complexes (2:1:1:1, modelled from 4GPO; 4:2:2:2, modelled from 4DKL; and 3:3:3:1:1:1, modelled from 4AY9 for the extracellular domain, while the transmembrane region was modelled with SWISS-MODEL [124] and M-ZDOCK [125]. (B) Asymmetric structures of dimeric receptors (PDB IDs: 4HSA, 3NPZ, 4CN2, 4IQR and 2GS6). (C) Symmetric and pseudosymmetric structures of transmembrane channels (PDB IDs: 4UWE, 4PE5, 4U5B and 4BOI).

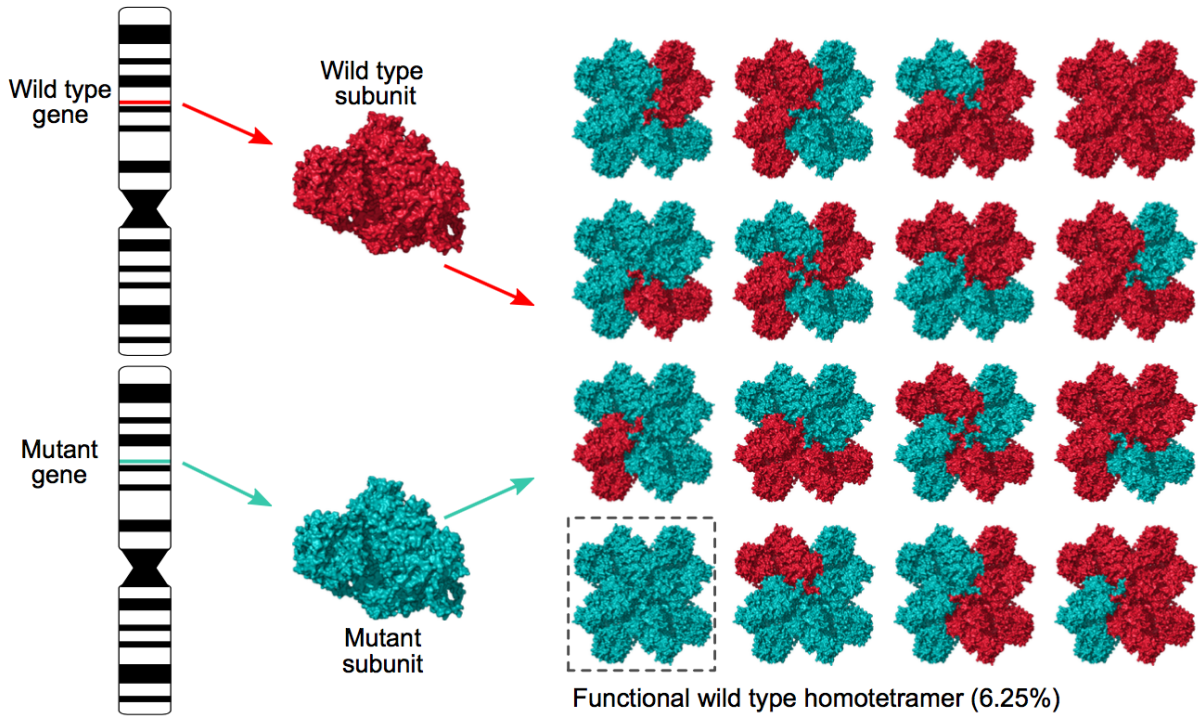

Figure 2: The dominant-negative effect in symmetric homomers.

Illustration of the dominant-negative effect using the structure of homotetrameric $InsP_3R1$ (PDB ID: 3JAV) [126], which is mutated in Gillespie syndrome and spinocerebellar ataxia [104,127]. In the case of heterozygous disease mutations and random association of proteins, 15/16 tetramers will contain at least one mutated subunit.

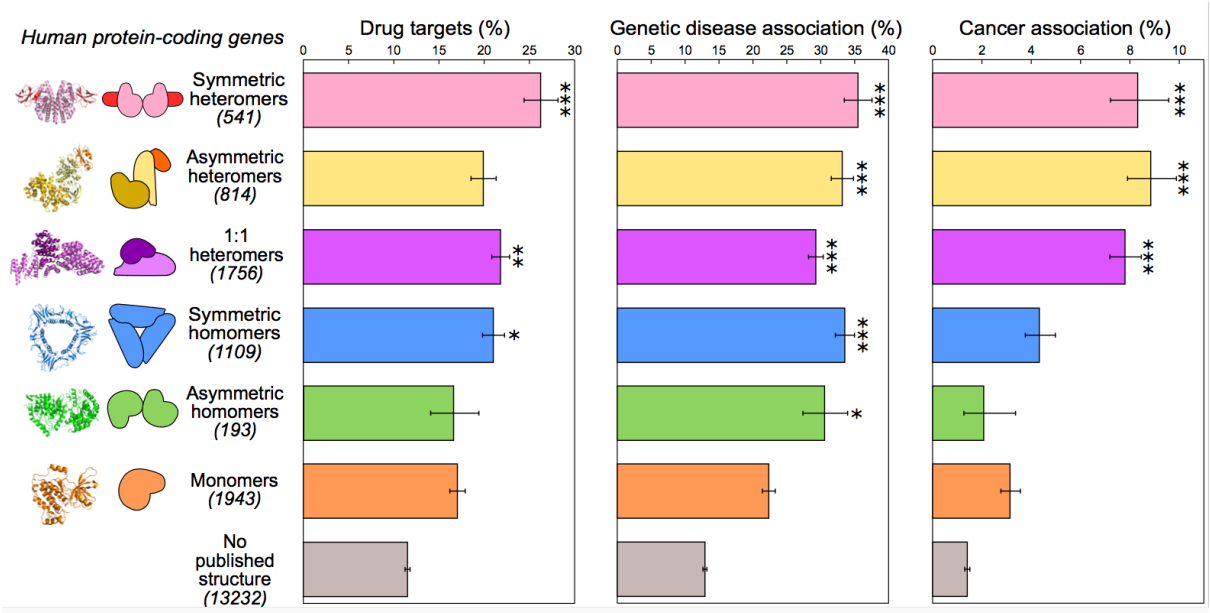

Figure 3: Structural bioinformatic analysis of the biomedical and pharmacological importance of protein complex symmetry and asymmetry.

Human protein-coding genes were classified based upon the type of protein structure formed in the Protein Data Bank. Drug target genes were taken from the Guide to Pharmacology [117]. Genes with a Mendelian genetic disease association were taken from OMIM [118]. Genes with a known cancer association were taken from COSMIC [119]. For human genes mapping (>70% sequence identity) to multiple structures, on a single quaternary structure classification was assigned: the highest category from top to bottom in the above plot (e.g. a gene mapping to a symmetric homomer and a monomer was classified as a symmetric homomer). *P*-values were calculated using Fisher's exact test comparing subunits from different types of complexes to monomers, indicated by * ($P \leq 0.01$), ** ($P \leq 0.0002$) and *** ($P \leq 3 \times 10^{-6}$). Error bars represent 68% Wilson binomial confidence intervals.

Table 1: Key examples of symmetry breaking in signalling complexes

Example	Description
GPCRs	Dimer symmetry can be broken through formation of uneven stoichiometry complexes with regulatory proteins or small molecules, or upon formation of higher-order oligomers.
Nuclear receptors	Many nuclear receptors are symmetric dimers, but this symmetry can be broken upon interaction with asymmetric double-stranded DNA.
EGFR	Forms a symmetric dimer in its autoinhibited state, but rearranges to an asymmetric dimer upon activation.
pLGICs	Mammalian heteropentamers formed from paralogous subunits are pseudosymmetric with uneven stoichiometry, while pseudosymmetry is broken upon activation by asymmetric ligand binding.
iGluRs	Homotetramer shows a mixture of C_2 and C_4 symmetry, which is broken upon accessory protein or toxin binding with uneven stoichiometry.